



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2392

Mitochondrial DNA Sequencing (Human)

This Standard Reference Material (SRM) is intended to provide quality control when performing the polymerase chain reaction (PCR) and sequencing of human mitochondrial DNA (mtDNA) for forensic identifications, medical diagnosis, or mutation detection. It may also be used as a control when amplifying (PCR) and sequencing any DNA. This SRM can also be used for quality assurance when assigning values to in-house control materials. It is certified for the sequences of the entire human mtDNA (16 569 base pairs) from two lymphoblastoid cell culture lines (CHR and GM09947A) from apparently normal individuals, plus the cloned HV1 region of CHR containing a C-stretch which is difficult to sequence. The SRM is packaged in a single box containing three components: (1) extracted DNA from cell culture line CHR (tube contains 60 µL of DNA at a concentration of 1 ng/µL); (2) extracted DNA from cell culture line GM09947A (tube contains 60 µL of DNA at a concentration of 1 ng/µL); and (3) cloned DNA from the CHR HV1 region containing the C-stretch (tube contains 10 µL of DNA at a concentration of 100 ng/µL).

This SRM is composed of well-characterized extracted human DNA from CHR and GM09947A and cloned DNA from the HV1 region of CHR. Table 1 contains the certified sequence information of two entire mtDNA templates (CHR and GM09947A). Table 2 contains the reference sequences of 58 unique primer sets which were designed to amplify any portion or the entire human mtDNA. The sequence information of a third DNA template (GM03798) that was amplified and sequenced in its entirety three to four times at NIST is provided in reference 1. Although the extracted DNA from GM03798 is not provided, the cell culture line can be obtained from NIGMS Human Genetic Mutant Cell Repository, Coriell Institute for Medical Research, Camden, NJ.

Expiration of Certification: The certification of this SRM is valid until **31 May 2008**, provided the SRM is handled and stored in accordance with the instructions given in this certificate. This certification is nullified if the SRM is damaged, contaminated, or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Return of the attached registration card will facilitate notification.

The overall direction and coordination of the technical measurements leading to the certification were performed by B.C. Levin and D.J. Reeder of the NIST DNA Technologies Group, Biotechnology Division.

The analytical determination and technical measurements for the certification of this SRM were performed by B.C. Levin, H. Cheng, L.A. Tully, M.P. Jones, and D.J. Reeder of the NIST DNA Technologies Group, Biotechnology Division.

The support aspects involved in the preparation, certification, and issuance of this SRM were coordinated through the NIST Standard Reference Materials Program by J.C. Colbert and B.S. MacDonald of the NIST Measurement Services Division.

Vincent L. Viker, Chief
Biotechnology Division

John Rumble, Jr., Chief
Measurement Services Division

Gaithersburg, MD 20899
Certificate Issue Date: 17 June 2003
See Certificate Revision History on Last Page

NOTICE AND WARNING TO USER

Warning: SRM 2392 IS A HUMAN SOURCE MATERIAL. SINCE THERE IS NO CONSENSUS ON THE INFECTIOUS STATUS OF EXTRACTED DNA, HANDLE PRODUCT AS A BIOHAZARDOUS MATERIAL CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.

Storage: Store frozen at a temperature of -20 °C. **DO NOT** use a self-defrosting freezer because periodic cycling of temperatures may cause shortened shelf life of this SRM.

INSTRUCTIONS FOR USE

It is recommended that once thawed, each SRM component should be used in its entirety. Repeated freezing and thawing is **NOT** recommended as this might shorten the shelf life of the SRM. If it is necessary to perform repeated analyses, thaw the SRM and divide the tube contents into aliquots that will be kept frozen until use. Thawing can be conducted at refrigerator temperatures, room temperature, or at 37 °C. Once thawed, the sample should be processed without delay.

SOURCE AND ANALYSIS¹

Source of Material: CHR DNA, both extracted and cloned, was prepared in the NIST DNA Technologies Group, Biotechnology Division. DNA for GM09947A was prepared by Life Technologies, Inc., Gaithersburg, MD.

NIST Analysis: NIST extracted DNA from the CHR cell culture, PCR was used to amplify both the CHR DNA and GM09947A DNA with all 58 primer sets multiple times, and sequenced the PCR products with a Perkin-Elmer Applied Biosystems, Inc. (ABI) 373 automated sequencer or an ABI 310 sequencer. The cloned DNA was prepared at NIST as described in reference 1. The sequence of the CHR clone and of representative PCR products of the final CHR and GM09947A DNA included in SRM 2392 was reanalyzed to ensure sequence accuracy.

Interlaboratory Analysis: An interlaboratory evaluation of the amplification, sequencing, and data analysis of the CHR template was conducted by four laboratories, including NIST. These laboratories were: The Bode Technology Group, Inc., Sterling, VA; IIT Research Institute, Virginia Technology Center, Newington, VA; and Lark Technologies, Inc., Houston, TX. Description of the interlaboratory analyses is described in reference 1.

Description of Components: Three components are included in each unit; all components must be stored at -20 °C.

- # 1 Extracted DNA from cell culture line CHR (tube contains 60 µL of DNA at a concentration of 1 ng/µL)
- # 2 Extracted DNA from cell culture line GM09947A (tube contains 60 µL of DNA at a concentration of 1 ng/µL)
- # 3 Cloned DNA from the CHR HV1 region containing the C-stretch (tube contains 10 µL of DNA at a concentration of 100 ng/µL)

NOTE: DNA concentrations given are nominal values and are not intended for use as concentration standards.

¹Certain commercial equipment, instruments, materials, or companies are identified in this paper to specify the experimental procedure. Such identification does not imply recommendation or endorsement by NIST, nor does it imply that the materials or equipment identified are the best available for this purpose.

Table 1. Certified Human mtDNA Sequence Differences from the Cambridge Reference Sequence [2] Found in Two Templates at NIST

Primer Set	Amplified Region ^a	Length of Amplified Region	Comparison with Cambridge Reference Sequence (CRS)					Amino Acid
			CRS #	bp	Template CHR	Template GM09947A	Change	
1 (HV2)	15 - 484	470	73 93 195 204 207 214 263 309.1 309.2 315.1	A A T T G A A C(ins) - C(ins)	Start 39 G - C C A - G G C(ins) C(ins) C(ins) End 436	Start 39 - G C - - G G C(ins) C(ins) C(ins) End 473		
2	361 - 921	561	709 750	G A	Start 429 A G End 891	Start 421 - G End 846		
3	756 - 1425	670	NONE		Start 778 End 1197	Start 778 End 1278		
4	873 - 1425	553	NONE		Start 931 End 1335	Start 928 End 1377		
5	1234 - 1769	536	1438 1719	A G	Start 1279 G A End 1738	Start 1275 G - End 1741		

Table 1. Continued

6	1587 - 2216	630	1719 ^d	G	Start 1632 A End 2106	Start 1632 - End 2106	
7	1657 - 2216	560	1719 ^d	G	Start 1691 A End 2170	Start 1686 - End 2173	
8	1993 - 2216	224	NONE		Start 2036 End 2213	Start 2018 End 2217	
9	2105 - 2660	556	NONE		Start 2157 End 2636	Start 2150 End 2586	
10	2417 - 3006	590	2706	A	Start 2465 G End 2920	Start 2458 - End 2956	
11	2834 - 3557	724	3106/3107	C	Start 2861 Del End 3350	Start 2869 Del End 3373	
12	2972 - 3557	586	3106/3107 ^d 3423	C G	Start 2999 Del E End 3422	Start 2999 Del T End 3460	Silent
13	3234 - 3557	324	3423 ^d	G	Start 3265 T End 3548	Start 3258 T End 3545	Silent ^d
14	3441 - 3940	500	NONE		Start 3487 End 3916	Start 3491 End 3920	
15	3635 - 4162	528	NONE		Start 3667 End 4126	Start 3662 End 4061	
16	3931 - 4728	798	4135	T	Start 3964 - End 4399	Start 3968 C End 4427	Try→His

Table 1. Continued

17	4183 - 4728	546	NONE		Start 4208 End 4657	Start 4249 End 4657	
18	4392 - 4982	591	4769	A	Start 4449 G End 4860	Start 4453 G End 4935	Silent
19	4447 - 4982	536	4769 ^d	A	Start 4492 G End 4958	Start 4492 G End 4921	Silent ^d
20	4797 - 5553	757	4985 5186	G A	Start 4838 A G End 5327	Start 4845 A - End 5324	Silent Silent
21	4976 - 5553	578	5186 ^d	A	Start 5000 G End 5516	Start 5007 - End 5521	Silent ^d
22	5318 - 5882	565	NONE		Start 5361 End 5754	Start 5360 End 5758	
23	5700 - 6262	563	NONE		Start 5741 End 6149	Start 5744 End 6163	
24	5999 - 6526	528	6221 6371	T C	Start 6043 C T End 6442	Start 6058 - - End 6503	Silent Silent
25	6242 - 6526	285	6371 ^d	C	Start 6271 T End 6520	Start 6302 - End 6520	Silent ^d
26	6426 - 7030	605	6791 6849*	A A	Start 6451 G G(0.3A) ^{h*} End 6916	Start 6474 - - End 6930	Silent Thr→Ala*

Table 1. Continued

27	6744 - 7255	512	6849 ^{d*} 7028	A C	Start 6775 G(0.3A) ^{h*} T End 7215	Start 6782 - End 7221	Thr→Ala ^{d*} Silent
28	7075 - 7792	718	NONE		Start 7123 End 7602	Start 7123 End 7601	
29	7215 - 7792	578	7645	T	Start 7263 - End 7722	Start 7280 C End 7769	Silent
30	7645 - 8215	571	7861	T	Start 7671 - End 8149	Start 7666 C End 8155	Silent
31	7901 - 8311	411	NONE		Start 7960 End 8289	Start 7959 End 8288	
32	8164 - 8669	506	8448 8503	T T	Start 8211 - C End 8646	Start 8212 C - End 8641	Met→Thr Silent
33	8539 - 9059	521	8860	A	Start 8581 G End 9019	Start 8582 G End 8999	Thr→Ala
34	8903 - 9403	501	9315	T	Start 8947 - End 9380	Start 8944 C End 9381	Phe→Leu
35	9309 - 9848	540	9559	G	Start 9334 C End 9823	Start 9333 C End 9827	Arg→Pro
36	9449 - 9995	547	9559 ^d	G	Start 9476 C End 9964	Start 9485 C End 9940	Arg→Pro ^d

Table 1. Continued

37	9754 - 10275	522	NONE		Start 9777 End 10225	Start 9781 End 10251	
38	10127 - 10556	430	NONE		Start 10168 End 10534	Start 10166 End 10536	
39	10386 - 11166	781	NONE		Start 10410 End 10899	Start 10416 End 10916	
40	10704 - 11267	564	NONE		Start 10734 End 11223	Start 10742 End 11197	
41	11001 - 11600	600	11335	T	Start 11026 C End 11461	Start 11040 C End 11517	Silent
42	11403 - 11927	525	11719	G	Start 11428 A End 11795	Start 11432 - End 11853	Silent
43	11760 - 12189	430	11878	T	Start 11784 C End 12159	Start 11802 - End 12164	Silent
44	11901 - 12876	976	NONE		Start 11926 End 12404	Start 11926 End 12443	
45	12357 - 12876	520	12612 12705	A C	Start 12404 G T End 12769	Start 12391 - - End 12849	Silent Silent
46	12601 - 13123	523	12705 ^d	C	Start 12627 T End 13102	Start 12645 - End 13045	Silent ^d
47	12793 - 13343	551	NONE		Start 12817 End 13295	Start 12807 End 13307	

Table 1. Continued

48	13188 - 13611	424	13572	T	Start 13238 - End 13587	Start 13238 C End 13593	Silent
49	13518 - 13935	418	13572 ^d 13702 13708 13759	T G G G	Start 13541 - C A - End 13910	Start 13541 C C - A End 13921	Silent ^d Gly→Arg Ala→Thr Ala→Thr
50	13715 - 14118	404	13966	A	Start 13775 G End 14094	Start 13760 - End 14110	Thr→Ala
51	13899 - 14388	490	13966 ^d 14199 14272 14365	A G G G	Start 13926 G T C C End 14369	Start 13927 - T C C End 14374	Thr→Ala ^d Pro→Thr Phe→Leu Silent
52	14189 - 14926	738	14272 ^d 14365 ^d 14368 14470 14766	G G G T T	Start 14216 C C C C E End 14699	Start 14216 C C C - C End 14806	Phe→Leu ^d Silent ^d Phe→Leu Silent Ile→Thr
53	14470 - 14996	527	14766 ^d	T	Start 14502 - End 14957	Start 14513 C End 14972	Ile→hr ^d
54	14909 - 15396	488	15326	A	Start 14941 G End 15380	Start 14933 G End 15373	Thr→Ala

Table 1. Continued

55	15260 - 15774	515	15326 ^d	A	Start 15305 G End 15754	Start 15293 G End 15950	Thr → Ala ^d
56	15574 - 16084	511	NONE		Start 15637 End 16056	Start 15599 End 16058	
57 (HV1)	15971 - 16451	481	16183 16189 16311	A T T	Start 16014 C C E End 16193	Start 16011 - - C End 16430	
58	16097 - 336	809	16183 ^d 16189 ^d 16311 ^d 16519	A T T T	Start 16125 C C E E End 16193	Start 16130 - - C C End 59	
-21M13 ^c cloned DNA	16133 - 40	477	16183 ^d 16189 ^d 16193.1 16223 16278 16519 ^d	A T C C T	Start 16131 C C C(ins) T T C End 40	ND	

Table 1. Continued

a	Numbers correspond to Cambridge Reference Sequence [2].
E	Base pair change came after the readable sequence.
-	Base pair same as in Cambridge Reference Sequence [2].
h*	Possible heteroplasmic site
*	This heteroplasmy seen in the first CHR cell culture line was not seen with the second CHR cell culture line. It is the second CHR cell culture line that is supplied in NIST SRM 2392.
c	This primer is used for sequencing the cloned DNA of the HV1 region.
d	Change also seen in previous primer set.
Start	Start of readable sequence.
End	End of readable sequence.
CHR cells	Sequence based on two amplifications and cycle sequencing procedures in first cell culture line and at least one amplification and cycle sequencing procedure with the second cell culture line.
GM09947A cells	Sequence based on two amplifications and cycle sequencing procedures.
Ins	Insertion
Del	Deletion
ND	Not done

Table 2. Reference Sequences for Primer Sets Used for PCR Amplification of Human mtDNA

PRIMER SET NUMBER	PRIMER SEQUENCE	
1 (HV2)	F15 R484	CACCCTATTAACCACACTCACG TGAGATTAGTAGTATGGGAG
2	F361 R921	ACAAAGAACCTAACACCAGC ACTTGGGTTAACCGTGTGACC
3	F756 R1425	CATCAAGCACGCAGCAATG AATCCACCTTCGACCCCTTAAG
4	F873 R1425	GGTTGGTCAATTCTGCCAG AATCCACCTTCGACCCCTTAAG
5	F1234 R1769	CTCACCCACCTCTGCTCAGC GCCAGGTTCAATTCTATCG
6	F1587 R2216	TGCACTTGGACGAACCAGAG TGGTGGAGCTTGAACGCTTC
7	F1657 R2216	CTTGACCGCTCTGAGCTAAC TGGTGGAGCTTGAACGCTTC
8	F1993 R2216	AAACCTACCGAGCCTGGTG TGGTGGAGCTTGAACGCTTC
9	F2105 R2660	GAGGAACAGCTTTGGACAC AGAGACAGCTGAACCTCGTG
10	F2417 R3006	CACTGTCAACCCAACACAGG ATGTCCTGATCCAACATCGAG
11	F2834 R3557	CCCAACCTCCGAGCAGTACATG AGAAGAGCGATGGTGAGAGC
12	F2972 R3557	ATAGGGTTACGACCTCGATG AGAAGAGCGATGGTGAGAGC
13	F3234 R3557	AGATGGCAGAGCCCGGTAAATC AGAAGAGCGATGGTGAGAGC
14	F3441 R3940	ACTACAACCTTCGCTGACG TGAAGCCTGAGACTAGTCGG
15	F3635 R4162	GCCTAGCCGTTACTCAATCC TGAGTTGGTCGTAGCGGAATC
16	F3931 R4728	TCAGGCTTCAACATCGAATACG TTATGGTTCATGTCCGGAGAG

Table 2. Continued

17	F4183 R4728	TTTCTACCACTCACCCTAGCATTAC TTATGGTTCATTGTCCGGAGAG
18	F4392 R4983	CCCATCCTAAAGTAAGGT CAGC GTTTAATCCACCTCAACTGCC
19	F4447 R4982	TTGGTTATAACCCTCCCGTAC GTTAATCCACCTCAACTGCC
20	F4797 R5553	CCCTTCACTTCTGAGTCCCAG AGGGCTTGAGGCTCTTG
21	F4976 R5553	ATTAACCAGACCCAGCTACG AGGGCTTGAGGCTCTTG
22	F5318 R5882	CACCATCACCCCTCCTAACCC GCTGAGTGAAGCATTGGACTG
23	F5700 R6262	TAAGCACCCATAATCAACTGGC GCCTCCACTATAGCAGATGCG
24	F5999 R6526	TCTAAGCCTCCTTATTCGAGC ATAGTGATGCCAGCAGCTAGG
25	F6242 R6526	CGCATCTGCTATAGTGGAGG ATAGTGATGCCAGCAGCTAGG
26	F6426 R7030	GCCATAACCAATACCAAACG TGGGCTACAACGTAGTACGTG
27	F6744 R7255	GGCTTCCTAGGGTTATCGTG TTTCATGTGGTGTATGCATCG
28	F7075 R7792	GAGGCTTCATTCACTGATTCC GGGCAGGATAGTCAGACGG
29	F7215 R7792	CGACGTTACTCGGACTACCC GGGCAGGATAGTCAGACGG
30	F7645 R8215	TATCACCTTCATGATCACGC GACGATGGGCATGAAACTG
31	F7901 R8311	TGAACCTACGAGTACACCGACTAC AAGTTAGCTTACAGTGGCTCTAG
32	F8164 R8669	CGGTCAATGCTCTGAAATCTGTG CATTGTTGGGTGGT GATTAGTCG
33	F8539 R9059	CTGTTCGCTTCATTCAATTGCC GTGGCGCTCCAATTAGGTG
34	F8903 R9403	CCCACTTCTTACCAAGGC GTGCTTTCTCGTGTACATCG

Table 2. Continued

35	F9309 R9848	TTTCACTTCACTCCATAACGC GAAAGTTGAGCCAATAATGACG
36	F9449 R9995	CGGGATAATCCTATTATTACCTCAG AGAGTAAGACCCTCATCAATAGATGG
37	F9754 R10275	AGTCTCCCTCACCATTCG AAAGGAGGGCAATTCTAGATC
38	F10127 R10556	ACTACCACAACCTAACGGCTAC GGAGGATATGAGGTGTGAGCG
39	F10386 R11166	GGATTAGACTGAACCGAATTGG CATCGGGTGATGATAGCCAAG
40	F10704 R11267	GTCTCAATCTCCAACACATATGG TGGTGTGAGTGTAAATTAGTGCG
41	F11001 R11600	AACGCCACTTATCCAGTGAACC CTGTTGTCGTAGGCAGATGG
42	F11403 R11927	GACTCCCTAAAGCCCCATGTCG TTGATCAGGAGAACGTGGTTAC
43	F11760 R12189	ACGAACGCACTCACAGTCG AAGCCTCTGTTGTCAGATTAC
44	F11901 R12876	TGCTAGTAACCACGTTCTGGTG GATATGCCGATACGGTTG
45	F12357 R12876	AACCACCCCTAACCCCTGACTTCC GATATGCCGATACGGTTG
46	F12601 R13123	TTCATCCCTGTAGCATTGTTCG AGCGGATGAGTAAGAAGATTCC
47	F12793 R13343	TTGCTCATCAGTTGATGATACG TTGAAGAAGGCCTGGGTACAG
48	F13188 R13611	CACTCTGTCGCAGCAGTATG TCGAGTGCTATAGGCCTTGTGTC
49	F13518 R13935	CATCATCGAAACCGCAAAC TGTGATGCTAGGGTAGAATCCG
50	F13715 R14118	GAAGCCTATTCGCAGGATTTC TGGGAAGAAGAAAGAGAGGAAG
51 ^a	F13899 R14388 R14388	TTCTCCAACATACTCGGATTTC TTAGCGATGGAGGTAGGATTG (Old Primer) TTAGCGATGGAGGTAGGATT <u>GG</u> (New Primer)
52	F14189 R14926	ACAAACAATGGTCAACCAAGTAAC TGAGGCGTCTGGTGAGTAGTGC

Table 2. Continued

53	F14470 R14996	TCCAAAGACAACCATCATTCC CGTGAAGGTAGCGGATGATTG
54	F14909 R15396	TACTCACCAAGACGCCTCAACCG TTATCGGAATGGGAGGTGATTG
55	F15260 R15774	AGTCCCACCCCTCACACGATTG ACTGGTTGTCCTCCGATTCAAGG
56	F15574 R16084	CGCCTACACAATTCTCCGATC CGGTTGTTGATGGGTGAGTC
57 (HV1)	F15971 R16451	TTAACTCCACCATTAGCACC GCGAGGAGAGTAGCACTCTTG
58	F16097 R336	TACATTACTGCCAGCCACCATG TTAAGTGCTGTGGCCAGAAG
-21M13	F	TGTAAAACGACGGCCAGT

^a The reverse primer of set 51 has been changed. One should use the new primer.

REFERENCES

- [1] Levin, B.C.; Cheng, H.; Reeder, D.J.; *Human Mitochondrial DNA Standard Reference Material for Quality Control in Forensic Identification, Medical Diagnosis, and Mutation Detection*; Genomics, Vol. 55, pp. 135-146 (1999).
- [2] Anderson, S.; Bankier, A.T.; Barrell, B.G.; deBruijn, M.H.L.; Coulson, A.R.; Drouin, J.; Eperon, I.C.; Nierlich, D.P.; Roe, B.A.; Sanger, F.; Schreier, P.H.; Smith, A.J.H.; Staden, R.; Young, I.G.; *Sequence and Organization of the Human Mitochondrial Genome*; Nature, Vol. 290, pp. 457-465 (1981).

Certificate Revision History: 17 June 2003 (This revision reports an extension in expiration date and replacement of reverse primer 51); 29 December 1999 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet <http://www.nist.gov/srm>.